CHARACTERISATION OF LIGNIN EXTRACTED FROM SIX MANGROVE SPECIES GROWN IN BANGLADESH

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Lignin from six mangrove species [namely, keora (*Sonneratia apetala*), gewa (*Excoecaria agallocha*), bine (*Avicennia alba*), sundri (*Heritiera fomes*), pashur (*Xyloccarpous mekongests*) and kakra (*Bruguiera gymnorhiza*)] was extracted by acidic dioxane. The isolated lignin was submitted to structural characterization employing spectroscopic techniques (FTIR, ^{1}H-NMR), as well as elemental analysis, molecular weight and methoxyl content determination. Results showed that the lignin of these mangrove species was mainly of the syringyl type (S), followed by the guaiacyl type (G). The polydispersity of the mangrove species lignin was very high. NMR spectra showed that the *erythro* protons (Hα) give a stronger peak at 6.01 ppm than the corresponding peak for the *threo* form at 6.09 ppm in bine, gewa, kakra and sundari lignins, while keora lignin showed almost equal intensity.

Keywords: dioxane lignin, erythro form, guaiacyl unit, mangrove species, molecular weight, syringyl unit

INTRODUCTION

Mangrove forests play an important role in stabilizing the shoreline and reducing the distressing impact of natural disasters, such as cyclones, tsunamis and hurricanes. Mangrove forests provide breeding and nursing grounds for marine and pelagic species, while also supplying food, medicine, fuel and building materials for local people. The Sundarbans mangrove forest offers coastal protection to millions of people in Bangladesh and India. Currently, the Sundarbans covers approximately 10,000 km^2, 40% of which is in India and the rest is in Bangladesh.[1] The Sundarbans is a unique and the largest contiguous natural mangrove forest in the world. It is situated in the southern part of Satkhira, Khulna and Bagerhat districts, the south-western region of Bangladesh. It has an area of about 601,700 hectares, which represents 4.13% of the country and 38.12% of the state forest land. Sundri (*Heritiera fomes*) is the characteristic species of the freshwater zone of the Sundarbans. The forest of the moderately saltwater zone is a mixture of gewa (*Excoecaria agallocha*) and sundry, with varying amounts of goran (*Ceriops decandra*) and other species. The forest in the saltwater zone is dominated by goran (*Ceriops decandra*), gewa (*Excoecaria agallocha*), passur (*Xylocarpus mekongensis*) and dhandal (*Xylocarpus granatum*).

In addition to the Sundarbans, plantations along the shore of the coastal districts of Bangladesh commenced in 1961-62 to protect lives and properties from natural calamities, such as cyclones and tidal bores, and to stabilize the newly accreted lands. This initiative has gained momentum since 1980-81 with the aid of development partners, and afforestation programs have been extended over the foreshore islands, embankments and along the open coast. *Sonneratia apetala* and *Avicennia officinalis* are the main species of the coastal plantation. The mangrove plantations in the coastal area have reached 196,000 ha.

For industrial application, thorough knowledge of these species is needed. Although the chemistry of mangrove wood has not been studied yet as extensively as that of most other wood species, preliminary investigations of the

chemical constituents have led to the possibility of several new applications. Very little information is available on the chemical composition of mangrove species. Mansyur *et al.*[2] studied the chemical composition of various species of mangrove woods and showed variation in lignin and pentosans contents and in solubility. Miles *et al.*[3] investigated the chemical constituents (such as salts, organic acids, carbohydrates, hydrocarbons, benzoquinone, naphthofurans, sesquiterpenes, triterpenes, alkaloids, flavonoids, polymers, sulfur derivatives and tannins) of mangrove plants and their potential application in medicine and agriculture. Singh *et al.*[4] explored chemi-mechanical pulping by the cold soda process of different mangrove wood chips, such as *Heritiera littoralis*, *Bruguieva conjugata* and *Rhizophora mucronata*. They obtained pulps with 75-84% screened yield, with strength properties higher than those of mechanical pulps obtained from the same species. For the first time, Mun *et al.*[5] comprehensively studied the chemical constituents of six mangrove species. This research group also showed the pulping potential of these six mangrove species and recommended them for packaging grade paper.[6]

Lignin is a phenyl-propanoid (C_9) polyphenol, mainly linked by carbon-carbon bonds and arylglycerol ether bonds between the monomeric phenolic *p*-coumaryl (H), coniferyl (G) and sinapyl alcohol (S) units. Isolation techniques of lignin from softwood and hardwood are available and its chemistry being much better known.[7] Lignin from hardwood, softwood and non-wood has been studied by numerous researchers.[8-10] Evtuguin *et al.*[11] conducted studies on plantation *Eucalyptus globulus* lignin and found it to be of the S/G type, with an extremely high proportion of syringyl (*S*) units (82-86%) and a minor proportion of *p*-hydrophenyl propane (*H*) units. Oliveira *et al.*[12] showed that banana plant leaf sheath lignin was of the HGS type, with a molar proportion of p-hydroxyphenyl (H)/guaiacyl (G)/syringyl (S) units of 12:25:63. Most of the H units in lignin are terminal phenolic coumarates linked to other lignin substructures by benzyl and Cγ-ester bonds, in contrast to ferulates, which are mainly ether linked to bulk lignin. Hardwood lignin from the tropical region was demonstrated to have lower content of alcoholic and phenolic hydroxyl groups than temperate hardwood lignin.[13-15] However, no study has been found on the lignin chemistry of mangrove species. The objective of this study was to isolate lignin from six mangrove species grown in the Sundabans by the acidic dioxane method. Isolated lignins were characterized by FTIR and ^{1}H-NMR spectroscopy, alkaline nitrobenzene oxidation, molecular weight determination, as well as elemental and methoxyl analyses.

EXPERIMENTAL

Raw materials

Wood chips of six mangrove species, namely, keora (*Sonneratia apetala*), gewa (*Excoecaria agallocha*), bine (*Avicennia alba*), sundri (*Heritiera fomes*) pashur (*Xyloccarpous mekongests*) and kakra (*Bruguiera gymnorhiza*), were ground (40/60 mesh) separately in a Wiley mill, extracted with alcohol-toluene solvent and dried in vacuum over P_2O_5.

Isolation of lignin

The extract free wood meals were refluxed with acidic dioxane (9:1) solution. The concentration of HCl in the dioxane solution was adjusted to 0.2 mole/L. The dioxane to wood meal ratio was 8:1. The wood meal was refluxed with dioxane solution for about 1 hour in N_2 atm. The N_2 flow was maintained at 50 mL/min. After completing the reflux time, the wood meal dioxane mixture was filtered in a Buckner funnel using filter no. 2. The residue was washed with dioxane solution (9:1). The dioxane solution was then neutralized by adding solid Na_2CO_3 and filtered. The filtrate was concentrated in a vacuum evaporator at 40 °C. Then, concentrated dioxane solution was added dropwise to water to precipitate lignin. The lignin precipitate was washed and dried in vacuum over P_2O_5.

Dried crude lignin was dissolved in dioxane (9:1), and again precipitated in ether with constant stirring by a magnetic bar. The precipitated pure lignin was dried in vacuum over P_2O_5 and weighed. The yield of dioxane lignin was calculated based on Klason lignin. The purity of dioxane lignin was determined by measuring Klason lignin.

Elemental analysis

C, H, O and N analyses were carried out in an analytical centre at Kyushu University, Japan. The methoxyl content in dioxane lignin was determined in accordance with Japan International Standard Methods (JIS P8013 1972).

Acetylation

Dioxane lignin (100 mg) was added in 1.5 mL of dry pyridine – acetic anhydride (1:1) and kept for 72 h. The solution was added to a 10-fold volume of ice-cold water, whereupon the acetylated sample was recovered as a precipitate, which was purified by successive washing with water and dried under vacuum over P_2O_5.

Molecular weight

The weight average (Mw) and number average molecular (Mn) weight of acetylated lignins from keora, gewa, bine, sundry, pashur and kakra were determined by GPC on a Sodex KF-802.5 column. The samples were dissolved in tetrahydrofuran (THF) and 10 µL was injected. The column was operated at 30 °C and eluted with THF at a flow rate of 1 mL/min. The column was calibrated using polystyrene standards.

Spectroscopic analyses

FTIR: IR spectra were recorded by using a Shimadzu FTIR spectrometer model 8201PC. The dried samples were embedded in KBr pellets in the concentration of about 1 mg/100 mg KBr. The spectra were recorded in the absorption band mode in the range of 4000-400 cm^{-1}.

^{1}H NMR: the spectra of lignin solutions (100 mg of acetylated lignin in 0.5 mL CDCl$_3$) were recorded in a Bruker 400 spectrometer. The solvent was used as internal standard (ppm 7.25). For quantification of protons, the signal in the specified regions of the spectrum were integrated with respect to a spectrum-wide baseline drawn at the level of the background noise, and the results were referred to the signal for methoxyl protons, whose average number per C$_9$ unit was established as described above.

RESULTS AND DISCUSSION

These mangrove species yielded 44.9-56.2% dioxane lignin (Table 1). The yields were better than in the case of the nalita wood lignins, using the same isolation method.[13] The acidic isolation conditions employed for the lignin extraction process are believed to result in the hydrolysis of the lignin-carbohydrate complex (LCC) linkages, allowing the release of lignin fragments into the aqueous dioxane solution from nalita wood.[16] The purity of these lignins was of 85-91%. The yield of the lignin isolated from these six mangrove species was lower than that of other lignocellulosics and annual plants.[12,17] This can be explained by a strong structural association of *in situ* lignin with polysaccharides.

Elemental analysis and methoxyl content determination

Elemental analysis and determination of functional groups are important to understand the chemical structure of isolated lignin. Chemical analyses were conducted on the lignin isolated from different mangrove species, and C$_9$ atoms and group ratios are shown in Table 2. The results show that the lignin from all of these mangrove species had almost similar C, H, N content, but the methoxyl content was the highest in gewa (22.0%) and the lowest in sundari (18.6%). The lignin sample showed a high content of methoxyl groups (1.33-1.63/C$_9$), which was close to that of *Eucalyptus globulus* wood.[11] Nitrogen was mainly derived from the protein–lignin complexes formed during delignification. The methoxyl content in these mangrove species was higher than that of other hardwood species.[18]

FTIR spectra

The band at 1328 cm^{-1} is associated with the C=O stretching of the syringyl structure in lignin molecules, whereas the band at 1030 cm^{-1}, which is characteristic of primary alcohol,[19] is found in all the mangrove species lignins. Strong bands at 1330, 1220 and 1120 cm^{-1} corresponding to the syringyl unit are found in all the lignins, whereas a small shoulder or none at all corresponding to the guaiacyl unit was found at 1275, 1153 and 1037 cm^{-1}.[20] These results are consistent with the findings reported for alkaline nitrobenzene oxidation.[5] The vibrations attributable to the aromatic rings are found at 1600 and 1510 cm^{-1}. A weak band at 1725 and 1650 cm^{-1}, due to either the acetyl or the uronic ester groups, were observed in all the mangrove species,[21] which indicated that the isolated lignin presented minor carbohydrate impurities.

Table 1
Lignin yields for six mangrove species

Sample	Lignin yield, % (based on Klason lignin)
Sundari	49.8
Bine	44.9
Keora	56.2
Gewa	47.6
Kakra	48.3
Pashur	55.5

Table 2

Elemental analysis and methoxyl content of six mangrove species lignins

Sample	Percentage (%)					C_9 formula[*]
	C	H	N	O	OCH$_3$	
Sundari	55.97	5.45	0.38	38.20	18.6	$C_9H_{8.09}O_{5.72}(OCH_3)_{1.33}$
Bine	55.62	5.98	0.26	38.14	21.7	$C_9H_{8.88}O_{5.67}(OCH_3)_{1.60}$
Keora	57.60	5.97	0.30	36.13	20.7	$C_9H_{8.64}O_{5.10}(OCH_3)_{1.45}$
Gewa	55.34	6.00	0.18	38.48	22.0	$C_9H_{8.93}O_{5.67}(OCH_3)_{1.63}$
Kakra	56.35	6.00	0.16	37.49	20.8	$C_9H_{8.92}O_{5.49}(OCH_3)_{1.50}$
Pashur	55.18	6.00	0.26	38.56	21.8	$C_9H_{8.88}O_{5.80}(OCH_3)_{1.62}$

[*]Empirical formula $C_xH_yO_z(OCH_3)_n$ was calculated as follows: $n = (\%OCH_3)/31.04$; $x = (\%C)/12 - n$; $y = (\%H) - 3n$; $z = (\%O)/16 - n$
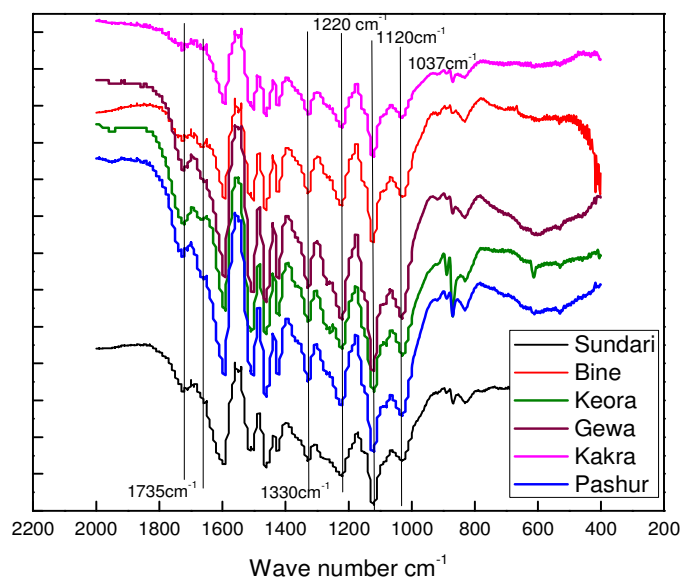

Figure 1: FT-IR spectra of six mangrove species lignins

The bands at around 1735 cm^{-1} and at 1370 cm^{-1} are due to the presence of acetyl groups.[22] A strong absorbance at 1737 cm^{-1} is assigned to C=O in unconjugated ketones, carbonyls and ester groups.[20]

The ratio of A_{1462}/A_{1595}, related to the amounts of methoxyl groups in lignins,[22] also indicated the presence of a higher S/V ratio. This result is consistent with that of our previous communication, where the alkali S/V ratio of 1.6-4.0 was obtained by alkaline nitrobenzene oxidation from these six mangrove species.[5]

Molecular weight

The weight average (Mw) and number average (Mn) molecular weight, as well as the polydispersity of the lignin from the different mangrove species were computed from their chromatograms and are shown in Table 3. The weight average molecular weight (Mw) of the six mangrove species was found to be 4400-11600. The data shows that the Mw of bine lignin was the highest among these six mangrove species. The Mw of softwood lignins seems to be of the order of 20000, whereas lower values have been reported for hardwood lignin.[23] According to Pouteau,[24] low molecular weight lignins exhibit good compatibility with polypropylene and show antioxidant activity. The number average molecular weight (Mn) was very low, consequently, polydispersity was very high. The high polydispersity of lignin may cause lower solubility in polymeric materials.[25] The lower Mn can be explained by the cleavage of β-O-4 linkages by acid hydrolysis during the isolation of dioxane lignin.[17] It can be stated that the lignin extracted from the mangrove species has higher polydispersity and, consequently, lower solubility in polymeric form than other hardwood lignins.

^{1}H-NMR

The ^{1}H-NMR spectra of the acetylated dioxane lignins obtained from the six mangrove species are shown in Figure 3. Subdivisions of the integration curves were made according to Lundquist.[26] The estimation of the various types of protons per C_9 structural unit has been conducted and described elsewhere.[13] Table 4 shows the subdivisions and distribution of protons

found in the present samples. However, strictly quantitative conclusions cannot be drawn regarding the numbers of hydroxyl groups and other protons because of the uncertainty in fixing the boundaries of the resonances and the base lines in the spectra.

Table 3
Molecular weight of six mangrove species lignins

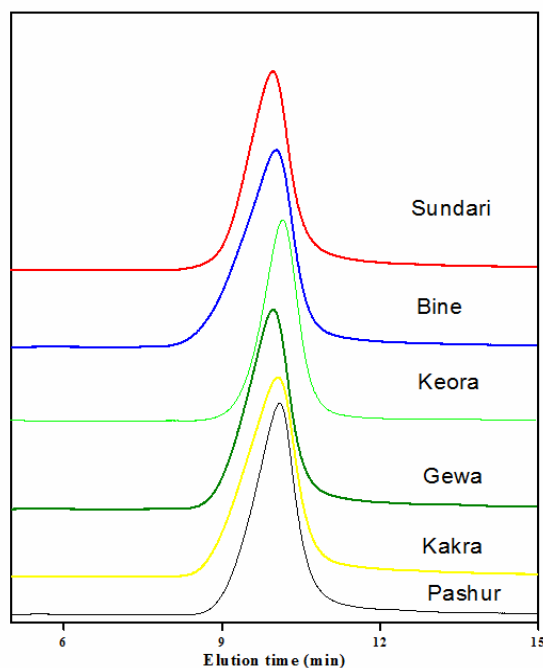| Sample	Mw	Mn	Mw/Mn
Sundari	6,822	318	21.5
Bine	11,629	438	26.5
Keora	4,469	399	11.2
Geoa	7,598	363	21
Kakra	8,473	355	23.9
Pashur	4,758	358	13.3

Figure 2: Molecular weight of six mangrove species lignins

As shown in the ^{1}H NMR spectra, higher syringyl propane content of the mangrove species lignin (Fig. 3) can be observed from the relative intensities of the resonances at 6.2-6.8 and 6.8-7.2 ppm, which can be assigned to the aromatic protons in syringyl propane and guaiacyl propane structures, respectively. Among the six mangrove species, keora lignin showed the lowest syringyl propane content. The quantification of the number of syringyl and guaiacyl protons per phenyl

propane unit was 1.06 and 0.55 for bine, 0.85 and 0.82 for keora, 0.97 and 0.58 for gewa, 0.75 and 0.39 for kakra, 0.94 and 0.65 pashur and 1.19 and 0.76 for sundri, respectively. These results suggest that the degree of condensation in kakra lignin is higher than in the other five mangrove species lignins.

From the compositional analysis, considering 100 C_9 units and their methoxyl contents, the syringyl and guaiacyl units contain 2 and 1 OCH_3

groups, respectively, in the lignin samples. For example, sundri lignin (1) $(x) + (2)(100 - x) = 133$, where X = no of guaiacyl units. In the same way, it was found that the proportion of guaiacyl to syringyl in sundri was 67% to 33%. Similarly, the following figures have been found: bine – 40 to 60%, keora – 55 to 45%, gewa – 37 to 63%, kakra – 50 to 50% and pashur – 38 to 62%.
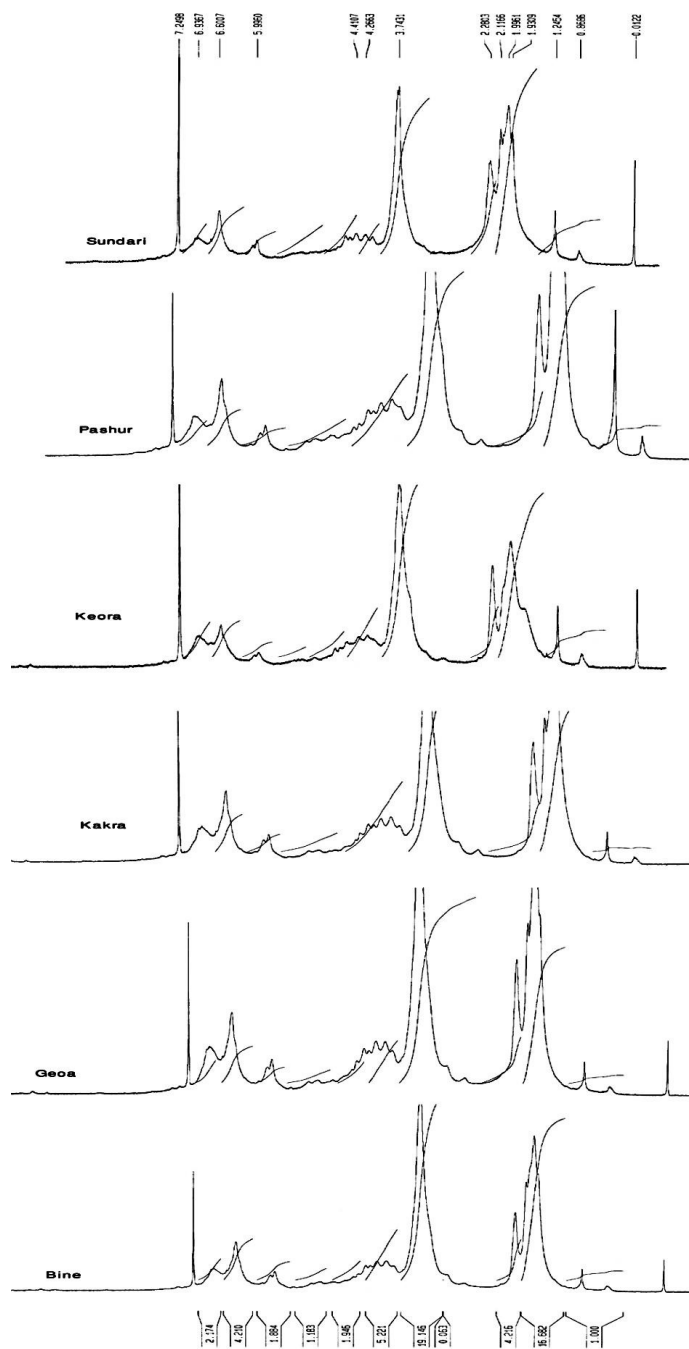

Figure 3: ^{1}H NMR spectra of dioxane lignins extracted from six mangrove species

Table 4

Assignments of signals and protons per C_9 structural unit in the ^{1}H NMR spectra of acetylated lignin of six mangrove species

Range ppm	Main assignments	Proton per C_9 unit					
		Pashur	Bine	Keora	Gewa	Kakra	Sundari
7.25-6.80	Aromatic proton in guaiacyl units	0.65	0.55	0.82	0.58	0.39	0.76
6.80-6.25	Aromatic proton in syringyl units	0.94	1.06	0.85	0.97	0.75	1.19
6.25-5.75	Hα of β-O-4 and β-1 structures	0.46	0.47	0.35	0.41	0.38	0.57
4.90-4.30	Hα & Hβ of β-O-4 structures	1.19	0.92	0.95	1.12	1.01	1.00
4.30-4.00	Hα of β-β structures H of xylan residue	0.70	1.31	1.15	0.56	0.40	0.78
4.00-3.48	H of methoxyl groups	4.86	4.80	4.35	4.89	3.75	3.99
2.50-2.22	H of aromatic acetates	1.44	1.05	1.20	1.23	0.96	1.89
2.22-1.60	H of aliphatic acetates	4.17	4.17	3.87	3.57	3.69	4.74

Considering both aliphatic and aromatic hydroxyls per C_9 unit (Table 4), the following calculation could be done: for example, for pashur lignin, the mole ratio OAc/OCH_3 = (1.44 + 4.17)/4.86 = 1.15. Thus, the total OAc/C9 ratio = (1.62 OCH_3) x (1.15 OAc/1 OCH_3) = 1.86; the number of aliphatic OAc/CH_3, = (1.62 OCH_3/C_9) X (4.17 OAc/4.86 OCH_3) = 1.39 and the number of aromatic OAc/CH_3, = (1.62) x (1.44/4.86) = 0.48. Therefore, the numbers of aliphatic and the free phenolic hydroxyls of pashur were estimated to be 139 and 48, respectively, per 100 C_9 units. Similarly, the numbers of aliphatic and phenolic hydroxyl groups were estimated to be 139 and 35 for bine, 129 and 40 for keora, 119 and 41 for gewa, 148 and 38 for kakra and 158 and 63 for sundri, respectively, per 100 C_9 units.

The main intermonomeric connections in lignins are aryl glycerol-β-O-4 aryl ether linkages.[26] The NMR spectra of the six mangrove species dioxane lignins show that the structural element may contain both *erythro* and *threo* configurations due to the presence of the proton at the C-α position of the side chain. The *erythro* protons (Hα) give a stronger peak at 6.01 ppm than the corresponding peak for the *threo* form at δ 6.09 in bine, gewa, kakra and sundri lignins, but keora lignin showed almost equal intensity (Fig. 3). The proton of the C-β and C-γ showed a peak at 4.6,[26] and the protons of these six mangrove species lignins were around 0.92-1.19/C_9.

CONCLUSION

The syringyl to guaiacyl ratios of the six mangrove species lignins investigated here were higher than in the case of other hardwoods. The polydispersity of these lignins was very high, which indicated lower solubility. The structural element contained both *erythro* and *threo* configurations due to the presence of the proton at the C-α position of the side chain. The *erythro* protons (Hα) gave a stronger peak at 6.01 ppm than the corresponding peak for the *threo* form at δ 6.09 in bine, gewa, kakra and sundri lignins, but keora lignin showed almost equal intensity.

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